

CONSTRUCT	MODULE A	MODULE B	MODULE C	MODULE D	VECTOR
pRN114	35S:LUC	--	<i>nos</i> :ZmWus2	CmYLCV:AtSTM	BeYDV (pCambia T-DNA)
pRN119	CmYLCV:LUC	--	<i>nos</i> :ZmWus2	35S:AtSTM	BeYDV (pCambia T-DNA)
pRN120	CmYLCV:LUC	--	<i>nos</i> :ZmWus2	AtUBQ10:AtSTM	BeYDV (pCambia T-DNA)
pRN227	35S:Cas9	AtU6:gRNA (NbPDS)	<i>nos</i> :ZmWus2	CmYLCV:AtSTM	BeYDV (pCambia T-DNA)
pMM113	35S:LUC	AtU6:gRNA (NbPDS)	<i>nos</i> :ZmWus2	CmYLCV:AtSTM	BeYDV (pCambia T-DNA)
pMM114	CmYLCV:LUC	AtU6:gRNA (NbPDS)	<i>nos</i> :ZmWus2	35S: <i>ipt</i>	BeYDV (pCambia T-DNA)
pMM117	35S:LUC	AtU6:gRNA (NbPDS)	Empty	Empty	BeYDV (pCambia T-DNA)
pMM131	35S:LUC	AtU6:gRNA (NbPDS)	Empty	CmYLCV:AtSTM	BeYDV (pCambia T-DNA)
pMM134	CmYLCV:LUC	AtU6:gRNA (NbPDS)	35S: <i>ipt</i>	Empty	BeYDV (pCambia T-DNA)
pMM135	CmYLCV:LUC	AtU6:gRNA (NbPDS)	<i>nos</i> :ZmWus2	Empty	BeYDV (pCambia T-DNA)
pMM136	CmYLCV:LUC	AtU6:gRNA (NbPDS)	empty	35S:MPΔ	BeYDV (pCambia T-DNA)
pMM146	CmYLCV:LUC	AtU6:gRNA (NbPDS)	ZmUbi1:ZmBBM	Empty	BeYDV (pCambia T-DNA)
pMM230	35S:AtCas9	AtU6:gRNA (VvMLO)	<i>nos</i> :ZmWus2	AtUBQ10:LUC	BeYDV (pCambia T-DNA)
pMM231	35S:AtCas9	AtU6:gRNA (VvMLO)	35S: <i>ipt</i>	AtUBQ10:LUC	BeYDV (pCambia T-DNA)
pMM232	35S:AtCas9	AtU6:gRNA (VvMLO)	AtUBQ10:LUC	35S:MPΔ	BeYDV (pCambia T-DNA)
pMM233	35S:AtCas9	AtU6:gRNA (VvMLO)	AtUBQ10:LUC	35S:STM	BeYDV (pCambia T-DNA)
pMM234	35S:AtCas9	AtU6:gRNA (VvMLO)	AtUBQ10:LUC	AtUbi10:BBM	BeYDV (pCambia T-DNA)
pMM235	35S:AtCas9	AtU6:gRNA (VvMLO)	AtUBQ10:LUC	Empty	BeYDV (pCambia T-DNA)
pMKV057	Empty	AtUBQ10:LUC	<i>nos</i> :ZmWus2	35S: <i>ipt</i>	BeYDV (pCambia T-DNA)
pMKV058	Empty	AtUBQ10:LUC	<i>nos</i> :ZmWus2	Empty	BeYDV (pCambia T-DNA)
pMKV059	Empty	AtUBQ10:LUC	Empty	35S: <i>ipt</i>	BeYDV (pCambia T-DNA)
pMKV060	Empty	AtUBQ10:LUC	Empty	Empty	BeYDV (pCambia T-DNA)

#### Supplementary Table 1

Constructs used in this study.

Note that the destination backbone can accept up to four Golden Gate cassettes, designated modules A-D.

Developmental Regulator Combinations	Constructs Co-Cultured	Starting Number of Seedlings	Total Number of Growths	Number of Shoot-like Growths	Number of White Shoot-like Growths	Full Plants Formed	Number of Edited Plants	Plants w/ Developmental Abnormalities
<i>BBM</i>	pMM146	24	0	0	0	0	0	0
<i>ipt</i>	pMM134	24	0	0	0	0	0	0
<i>MPΔ</i>	pMM136	24	0	0	0	0	0	0
<i>STM</i>	pMM131	24	0	0	0	0	0	0
<i>Wus2</i>	pMM135	24	20	4	0	1	1	1
All	pMM131, 134, 135, 136, 146	30	17	4	0	1	0	1
<i>BBM &amp; ipt</i>	pMM134,146	36	0	0	0	0	0	0
<i>BBM &amp; Wus2</i>	pMM135,146	31	12	4	0	3	0	0
<i>IPT &amp; MPΔ</i>	pMM134,136	34	0	0	0	0	0	0
<i>STM &amp; MPΔ</i>	pMM131,136	29	0	0	0	0	0	0
<i>Wus2 &amp; ipt</i>	pMM134,135	27	46	23	3.5	11	2	0
<i>Wus2 &amp; STM</i>	pMM131,135	36	29	17	3	11	2	7

**Supplementary Table 2**

Assessing developmental regulators for meristem induction by Fast-TrACC.

Individual T-DNA constructs were created containing luciferase, a gRNA for *PDS* and a developmental regulator. The regulators include: *nos:ZmWus2*, *ZmUb1:ZmBbm*, *CmYLCV:STM*, *35S:MPΔ* and *35S:ipt*. *A tumefaciens* strains, each with a given regulator, were pooled and delivered to seedlings of a transgenic line of *N. benthamiana* expressing Cas9. Seedlings were monitored for the formation of growths, formation of shoot-like growths and the generation of full plants. From the combinations tested, only five generated shoot-like growths that could be converted to full plants. Of these, two combinations were the most effective at generating full plants (*Wus2 & ipt* and *Wus2 & STM*). The data shown here is summarized in Fig. 1f, and editing outcomes are presented in Supplementary Fig. 4.

Seedling number	Phenotype	PDS1	PDS2
Parental Tissue	WT	-3bp, WT	WT (100%)
S1	WT	WT (100%)	WT (100%)
S3	WT	WT (100%)	WT (100%)
S5	WT	WT (48%), -3bp (46%)	WT (98%)
S7	WT	WT (48%) and -3bp (45%)	WT (99%)
S9	WT	WT (100%)	WT (96%)
S11	WT	WT (46%), -3bp (48%)	WT (99%)
S13	WT	-3bp (99.6%)	WT (99%)
S15	WT	WT (43%), -3bp (55%)	WT (97%)
S17	WT	-3bp (99.4%)	WT (100%)
S19	WT	-3bp (99%)	WT (98%)

**Supplementary Table 3**

*PDS2* mutations transmitted to the next generation by plant 5-14-1-8.

See also the Supplementary Data Set for DNA sequences for all mutations recovered.

Description	Sequence
PDS2 forward primer for NGS	GACTGGAGTTCAGACGTGCTTCCGATCTNNNTGGAACTGAAAGTCAGATGTT
PDS2 reverse primer for NGS	ACACTCTTCCCTACACGACGCTTCCGATCTNNNAATGGCAAGATACATCTC
PDS1 forward primer NGS	ACACTCTTCCCTACACGACGCTTCCGATCTNNNTGCCGTTAATTGAGAGTCC
PDS1 reverse primer for NGS	GACTGGAGTTCAGACGTGCTTCCGATCTNNNATGCTCGTATCAAATTC
PDS1 forward primer for Sanger sequencing	TGGGAAGTCAAAGATGGTC
PDS1 reverse primer for Sanger sequencing	ACAATAATGGGATGGGCCTGG
PDS2 forward primer for Sanger sequencing	TGGGAAGTCAAAGATGTT
PDS2 reverse primer for Sanger sequencing	CAAAGCTAGCTTGAGGTGAAGC
sgRNA target sequence in both PDS homologs	TTGGTAGTAGCGACTCCATG

#### Supplementary Table 4

Primer sequences used for PCR and DNA sequencing as well as the sgRNA target site.